SureSelect^{XT} Target Enrichment System for SOLiD 4 Multiplexed Sequencing

Protocol

Version 1.2, February 2012

SureSelect platform manufactured with Agilent SurePrint Technology

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In this Guide...

This guide describes the recommended operational procedures to capture genomic regions of interest using the Agilent SureSelect^{XT} Target Enrichment System Kit for SOLiD Multiplex Sequencing. This protocol is specifically developed and optimized to use Biotinylated RNA oligomer libraries, or Bait, to enrich targeted regions of the genome from repetitive sequences and sequences unrelated to the research focus.

1 Before You Begin

This chapter contains information (such as procedural notes, safety information, required reagents and equipment) that you should read and understand before you start an experiment.

2 Sample Preparation

This chapter contains instructions for prepped library production specific to the Life Technologies SOLiD System.

3 Hybridization

This chapter describes the steps to combine the prepped library with the blocking agents and the SureSelect capture library.

4 Addition of Barcode Tags by Post-Hybridization Amplification

This chapter describes the steps to amplify, purify, quantify, and pool the barcoded sample libraries after target enrichment hybridization.

5 Reference

This chapter contains reference information.

What's New in 1.2

- New product configuration and product numbers for SureSelect reagent kits and capture libraries, up to 24 Mb.
- Support for the optional use of the Agilent 2200 TapeStation for DNA quantitation and qualification.
- Support for SureSelect Human All Exon v4 and All Exon v4+UTRs capture libraries.
- The volume of barcode library to use for one reaction is clarified.

What's New in 1.1

- Support for large custom captures (up to 34 MB).
- Reagent cap colors are listed where available.
- More details given for the reagent kits to use for each step.
- Update to Covaris shearing volume.
- Update to solution storage information for some stopping points.

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1 Before You Begin

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Make sure you have the most current protocol. Go to the SureSelect Related Literature page on genomics.agilent.com and search for manual number G7530-90001.

Make sure you read and understand the information in this chapter and have the necessary equipment and reagents listed before you start an experiment.

NOTE Agilent cannot guarantee the SureSelect Target Enrichment kits and cannot provide technical support for the use of non-Agilent protocols to process samples for enrichment.

Procedural Notes

- Prolonged exposure of SureSelect Elution Buffer to air can decrease product performance by altering the pH of the solution. Keep the Elution Buffer container tightly sealed when not in use.
- To prevent contamination of reagents by nucleases, always wear powder-free laboratory gloves and use dedicated solutions and pipettors with nuclease-free aerosol-resistant tips.
- Maintain a clean work area.
- Do not mix stock solutions and reactions containing gDNA on a vortex mixer. Instead, gently tap the tube with your finger to mix the sample.
- Avoid repeated freeze-thaw cycles of stock and diluted gDNA solutions.
- When preparing frozen reagent stock solutions for use:
  1. Thaw the aliquot as rapidly as possible without heating above room temperature.
  2. Mix briefly on a vortex mixer, then spin in a centrifuge for 5 to 10 seconds to drive the contents off of walls and lid.
  3. Store on ice or in a cold block until use.
- In general, follow Biosafety Level 1 (BL1) safety rules.

Safety Notes

CAUTION Wear appropriate personal protective equipment (PPE) when working in the laboratory.

Required Reagents

Table 1 Required Reagents for Library Prep and Post-Hybridization Amplification

Description	Vendor and part number
DNA 1000 Kit	Agilent p/n 5067-1504
High Sensitivity DNA Kit	Agilent p/n 5067-4626
QPCR NGS Library Quantification Kit (SOLiD)	Agilent p/n G4881A
Herculase II Fusion DNA Polymerase (includes dNTP mix and 5x Buffer) 200 reactions 400 reactions (Each library requires 4 reactions for pre-capture amplification and 2 reactions for post-capture amplification.)	Agilent p/n 600677 p/n 600679
Nuclease-free Water (not DEPC-treated)	Ambion Cat #AM9930
Agencourt AMPure XP Kit 5 mL 60 mL 450 mL	Beckman Coulter Genomics p/n A63880 p/n A63881 p/n A63882
1X Low TE Buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)	Life Technologies p/n 4389764
E-Gel SizeSelect 2% Agarose Gel	Life Technologies p/n G6610-02
Qubit dsDNA HS Assay Kit *or* Qubit dsDNA BR Assay Kit 100 assays, 2-1000 ng 500 assays, 2-1000 ng 1000 assays, 2-1000 ng	Life Technologies p/n Q32851 Life Technologies p/n Q32850 Life Technologies p/n Q32853 Life Technologies p/n Q33130
Qubit assay tubes	Life Technologies p/n Q32856
100% Ethanol, molecular biology grade	Sigma-Aldrich p/n E7023

Table 2 SureSelect Reagent Kit

Reagent Kits	16 Reactions	96 Reactions	480 Reactions
SureSelect^{XT} Reagent Kit, S4	G9614A	G9614B	G9614C

Table 3 SureSelect Capture Library (select one)

Capture Libraries	16 Reactions	96 Reactions	480 Reactions
SureSelect^{XT} Human All Exon 50Mb	5190-4626	5190-4627	5190-4629
SureSelect^{XT} Human All Exon V4	5190-4631	5190-4632	5190-4634
SureSelect^{XT} Human All Exon V4+UTRs	5190-4636	5190-4637	5190-4639
SureSelect^{XT} Mouse All Exon	5190-4641	5190-4642	5190-4644
SureSelect^{XT} DNA Kinome	5190-4646	5190-4647	5190-4649
SureSelect^{XT} X-chromosome	5190-4651	5190-4652	5190-4653
SureSelect^{XT} Custom 1 kb up to 499 Kb	5190-4806	5190-4807	5190-4809
(reorder)	5190-4811	5190-4812	5190-4814
SureSelect^{XT} Custom 0.5 Mb up to 2.9 Mb	5190-4816	5190-4817	5190-4819
(reorder)	5190-4821	5190-4822	5190-4824
SureSelect^{XT} Custom 3 Mb up to 5.9 Mb	5190-4826	5190-4827	5190-4829
(reorder)	5190-4831	5190-4832	5190-4834
SureSelect^{XT} Custom 6 Mb up to 11.9 Mb	5190-4836	5190-4837	5190-4839
(reorder)	5190-4841	5190-4842	5190-4844
SureSelect^{XT} Custom 12 Mb up to 24 Mb	5190-4896	5190-4897	5190-4899
(reorder)	5190-4901	5190-4902	5190-4904

Table 4 Required Reagents for Hybridization

Description	Vendor and part number
Dynabeads MyOne Streptavidin T1	Life Technologies
2 mL	Cat #65601
10 mL	Cat #65602
100 mL	Cat #65603
Nuclease-free Water (not DEPC-treated)	Ambion Cat #AM9930

Optional Reagents

Table 5 Optional Reagents

Description	Vendor and part number
SureSelect gDNA Extraction Kit	
50 reaction kit	Agilent p/n G7505A
250 reaction kit	Agilent p/n G7505B

Required Equipment

Table 6 Required Equipment for Library Prep and Post-Hybridization Amplification

Description	Vendor and part number
2100 Bioanalyzer	Agilent p/n G2938C
Mx3005P Real-Time PCR System	Agilent p/n 401449 or equivalent
Thermal cycler	Agilent SureCycler, Life Technologies Veriti Thermal Cycler, BioRad (MJ Research) DNA Engine PTC-200, or equivalent
Covaris S-series Single Tube Sample Preparation System, Model S2	Covaris
Covaris microTUBE with AFA fiber and snap cap	Covaris p/n 520045
Eppendorf Microcentrifuge Model 5417R	Eppendorf p/n 022621807 (120 V/60 Hz), Eppendorf p/n 022621840 (230 V/50 Hz) or equivalent
Eppendorf fixed-angle rotor with standard lid	Eppendorf p/n 022636006
DNA LoBind Tubes, 1.5-mL PCR clean, 250 pieces	Eppendorf p/n 022431021 or equivalent
E-Gel iBase and E-Gel Safe Imager Combo Kit	Life Technologies p/n G6465
Qubit Fluorometer	Life Technologies p/n Q32857
E-Gel iBase and E-Gel Safe Imager Combo Kit or	Life Technologies p/n G6465
Safe Imager Real-Time Transilluminator *and*	Life Technologies p/n G6500
E-Gel iBase Power System	Life Technologies p/n G6400
Dynal DynaMag-2 magnetic stand	Life Technologies p/n 123-21D or equivalent
P10, P20, P200 and P1000 pipettes	Pipetman P10, P20, P200, P1000 or equivalent
Vacuum concentrator	Savant SpeedVac or equivalent
Ice bucket	

Table 6 Required Equipment for Library Prep and Post-Hybridization Amplification

Description	Vendor and part number
Powder-free gloves	
Sterile, nuclease-free aerosol barrier pipette tips	
Timer	
Vortex mixer	
Heat block at 37°C	

Table 7 Required Equipment for Hybridization

Description	Vendor and part number
Mx3000P/Mx3005P 96-well tube plates	Agilent p/n 410088 or equivalent
Mx3000P/Mx3005P optical strip caps	Agilent p/n 401425 or equivalent
MicroAmp Clear Adhesive Film	Life Technologies p/n 4306311 or equivalent
BD Clay Adams Nutator Mixer	BD Diagnostics p/n 421105 or equivalent
Dynal DynaMag-2 magnetic stand	Life Technologies p/n 123-21D or equivalent
P10, P20, P200 and P1000 pipettes	Pipetman P10, P20, P200, P1000 or equivalent
Pipet-Light Multichannel Pipette, 12 channels	Rainin p/n L12-20 or equivalent
Sterile, nuclease-free aerosol barrier pipette tips	
Thermal cycler	Agilent SureCycler, Life Technologies Veriti Thermal Cycler, BioRad (MJ Research) DNA Engine PTC-200, or equivalent
Timer	
Vortex mixer	
Water bath or heat block set to 65°C	

Optional Equipment

Table 8 Optional Equipment for Hybridization

Description	Vendor and part number
Tube-strip capping tool	Agilent p/n 410099

Table 9 Optional Equipment for Library Prep and Post-Hybridization Amplification

Description	Vendor and part number
2200 TapeStation System	Agilent p/n G2964AA or G2965AA
D1K ScreenTape	Agilent p/n 5067-5361
D1K Reagents	Agilent p/n 5067-5362

2 Sample Preparation

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This chapter contains instructions for prepped library production specific to the Applied Biosystems SOLiD System.

Before you begin, you can use the SureSelect gDNA Extraction Kit to extract genomic DNA. Refer to the *gDNA Extraction Kit Protocol* (p/n 5012-8701).

NOTE Make sure genomic DNA samples are of high quality with an OD 260/280 ratio ranging from 1.8 to 2.0.

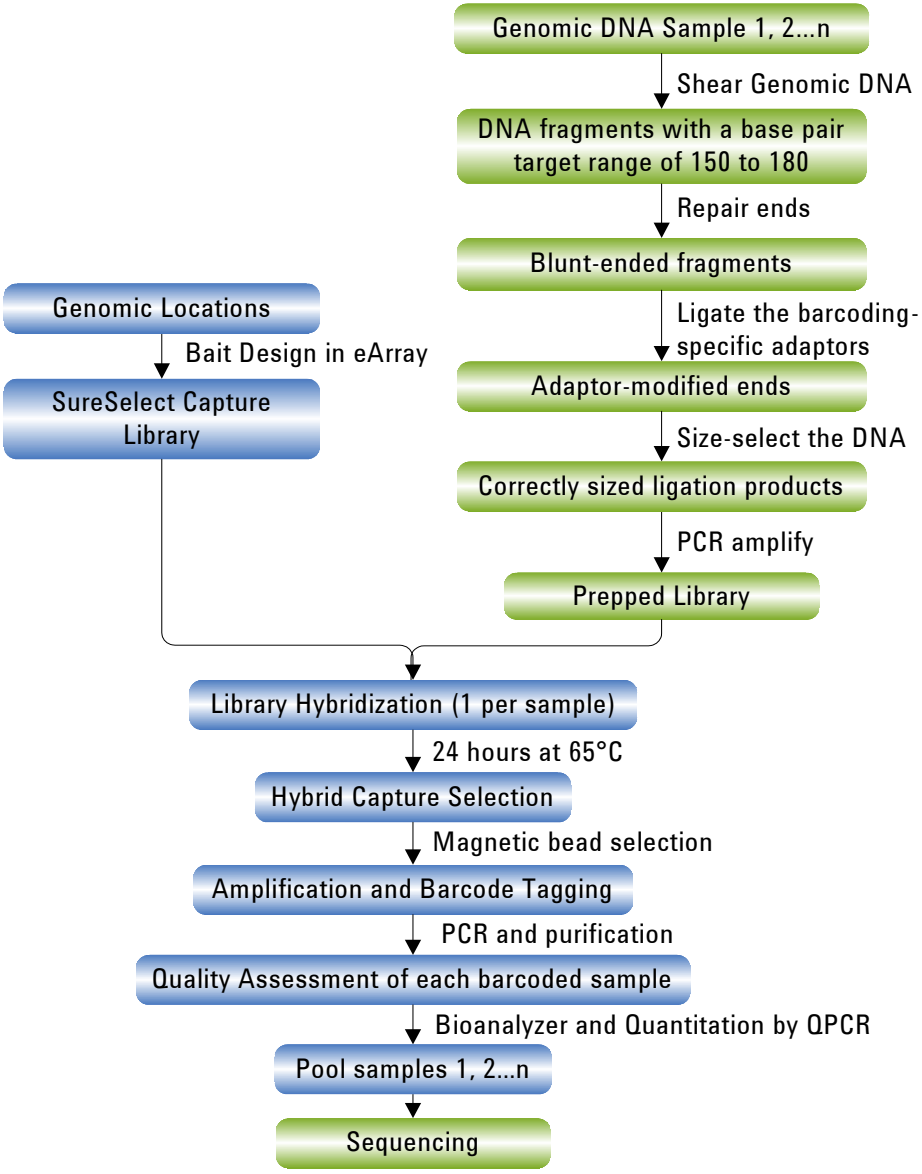

Figure 1 Overall sequencing sample preparation workflow.

Table 10 Overview and time requirements

Step	Time
AB SOLiD Fragment Library Production	8 hours
Bioanalyzer QC	1 hour
Hybridization	24 hours (optional 72 hours)
Bead Preparation	10 minutes
Capture Selection and Washing	2.5 hours
DNA Purification	30 minutes
Post-Hybridization Amplification	1 hour
PCR Purification	30 minutes
Bioanalyzer QC	1 hour

Step 1. Quantify and shear DNA

1. Use the Qubit dsDNA Assay to determine the concentration of your gDNA sample. Make sure the gDNA is of high quality (non-degraded, A_{260}/A_{280} is 1.8 to 2.0).

   Follow the instructions for the instrument.

2. Set up the Covaris instrument.

   a. Check that the water in the Covaris tank is filled with fresh deionized water to fill line level 12 on the graduated fill line label.

   b. Check that the water covers the visible glass part of the tube.

   c. Set the chiller temperature to between 2°C to 5°C to ensure that the temperature reading in the water bath displays 5°C.

   d. *Optional.* Supplement the circulated water chiller with ethylene glycol to 20% volume to prevent freezing.

   e. On the instrument control panel, push the Degas button. Degas the instrument for least 30 minutes before use.

   Refer to the Covaris instrument user guide.

3. Dilute 3 µg of high-quality gDNA with 1X Low TE Buffer in a 1.5-mL LoBind tube to a total volume of 130 µL.

4. Put a Covaris microTube into the loading and unloading station.

   Keep the cap on the tube.

5. Use a tapered pipette tip to slowly transfer the 130 µL DNA sample through the pre-split septa.

   Be careful not to introduce a bubble into the bottom of the tube.

6. Secure the microTube in the tube holder and shear the DNA with the settings in Table 11. The target peak for base pair size is 150 to 180 bp.

Table 11 Covaris shear settings

Setting	Value
Number of Cycles	6
Bath Temperature	5°C
Bath Temperature Limit	8°C
Mode	Frequency sweeping
Water Quality Testing Function	Off
Duty Cycle	10%
Intensity	5
Cycles per Burst	100
Time	60 seconds

7 Put the Covaris microTube back into the loading and unloading station.

8 While keeping the snap-cap on, insert a pipette tip through the pre-split septa, then slowly remove the sheared DNA.

9 Transfer the sheared DNA into a new 1.5-mL LoBind tube.

10 *Optional.* Quantify the sheared DNA with the 2100 Bioanalyzer DNA 1000 assay. See "Step 10. Quantify the Library with the 2100 Bioanalyzer DNA 1000 assay" on page 31 for instructions.

See Figure 2 on page 20.

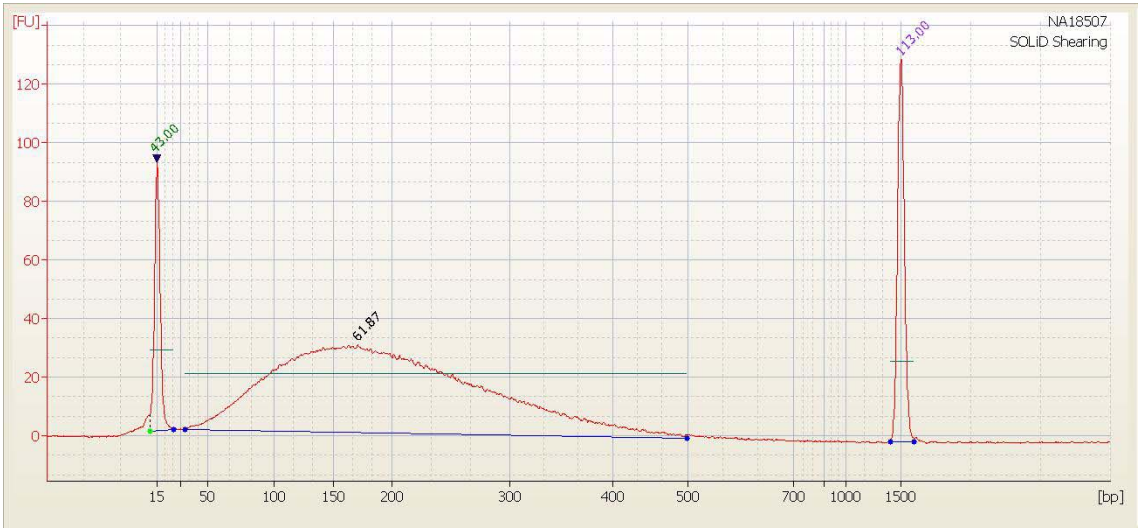

Figure 2 Analysis of sheared DNA using a DNA 1000 assay. The electropherogram shows a peak in the size range of approximately 50bp to 500bp.

Step 2. Purify the sample using the Agencourt AMPure XP beads

1. Measure out just enough AMPure XP beads for the number of samples to purify.
2. Let the AMPure XP beads come to room temperature for at least 30 minutes.
3. Mix the reagent well so that the reagent appears homogeneous and consistent in color. *Do not freeze*.
4. Add 180 µL of homogenous AMPure XP beads to a 1.5-mL LoBind tube, and add the sheared DNA library (~130 µL). Mix well on a vortex mixer and incubate for 5 minutes.
5. Put the tube in the magnetic stand. Wait for the solution to clear (approximately 3 to 5 minutes).
6. Keep the tube in the magnetic stand. Do not touch the beads while you carefully discard the cleared solution from the tubes.
7. Continue to keep the tube in the magnetic stand while you dispense 0.5 mL of 70% ethanol in each tube.

   Use fresh 70% ethanol for optimal result.
8. Let the tube sit for 1 minute to allow any disturbed beads to settle, and remove the ethanol.
9. Repeat step 7 and step 8 once.
10. Dry the samples on the 37°C heat block for 5 minutes until the residual ethanol is completely evaporated.

    Do not dry the bead pellet to the point that the bead pellet appears cracked. Elution efficiency is significantly decreased when the bead pellet is excessively dried.
11. Add 30 µL nuclease-free water, mix well on a vortex mixer, and incubate for 2 minutes at room temperature.
12. Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
13. Remove the supernatant (~30 µL) to a fresh 1.5-mL LoBind tube. You can discard the beads at this time.

Stopping Point If you do not continue to the next step, store the samples at -20°C.

Step 3. Repair the ends

Use reagents from the SureSelect Library Prep Kit, S4.

1 For 1 library:
   - In a 1.5-mL LoBind tube, strip tube, or plate, prepare the reaction mix in Table 12, on ice. Mix well by gently pipetting up and down.

2 For multiple libraries:
   a Prepare the reaction mix in Table 12, on ice. Mix gently on a vortex mixer.
   b Add 71 µL of the reaction mix to each well or tube.
   c Use a pipette to add 29 µL of each DNA sample to each well or tube. Mix by pipetting. Change pipette tips between samples.

Table 12 End Repair

Reagent	Volume for 1 Library (µL)	Volume for 12 Libraries (µL), includes excess
Sheared DNA	~29	
10X End Repair Buffer (clear cap)	10	125
dNTP mix (green cap)	1.6	20
T4 DNA Polymerase (purple cap)	1	12.5
Klenow DNA Polymerase (yellow cap)	1	12.5
T4 Polynucleotide Kinase (orange cap)	1.7	21.25
Nuclease-free water	55.7	696.25
Total Volume	**100**	**887.5 (71 µL/sample)**

3 Incubate the mixture at room temperature for 30 minutes.

Step 4. Purify the sample using the Agencourt AMPure XP beads

1. Let the AMPure XP beads come to room temperature for at least 30 minutes.
2. Mix the reagent well so that the reagent appears homogeneous and consistent in color. *Do not freeze*.
3. Add 180 µL of homogenous AMPure XP beads to a 1.5-mL LoBind tube, and add the repaired DNA library (100 µL). Mix well on a vortex mixer and incubate for 5 minutes.
4. Put the tube in the magnetic stand. Wait for the solution to clear (approximately 3 to 5 minutes).
5. Keep the tube in the magnetic stand. Do not touch the beads while you carefully discard the cleared solution from the tubes.
6. Continue to keep the tube in the magnetic stand while you dispense 0.5 mL of 70% ethanol in each tube.

   Use fresh 70% ethanol for optimal result.
7. Let the tube sit for 1 minute to allow any disturbed beads to settle, and remove the ethanol.
8. Repeat step 6 and step 7 once.
9. Dry the samples on the 37°C heat block for 5 minutes until the residual ethanol is completely evaporated.

   Do not dry the bead pellet to the point that the bead pellet appears cracked. Elution efficiency is significantly decreased when the bead pellet is excessively dried.
10. Add 25 µL nuclease-free water, mix well on a vortex mixer, and incubate for 2 minutes at room temperature.
11. Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
12. Remove the supernatant (~25 µL) to a fresh 1.5-mL LoBind tube. You can discard the beads at this time.

Stopping Point If you do not continue to the next step, store the samples at -20°C.

Step 5. Ligate the P1 and IA adaptors to the DNA

Use reagents from the SureSelect Library Prep Kit, S4 and the SureSelect LT Barcoding Lib. Kit.

1 For 1 library:
   - In a 1.5-mL LoBind tube, strip tube, or plate, prepare the reaction mix in Table 13, on ice. Mix well by gently pipetting up and down.

2 For multiple libraries:
   a Prepare the reaction mix in Table 13, on ice. Mix gently on a vortex mixer.
   b Add 76 µL of the reaction mix to each well or tube.
   c Use a pipette to add 24 µL of each DNA sample to each well or tube. Mix by pipetting. Change pipette tips between samples.

Table 13 Ligation mix

Component	Volume (µL)	Volume for 12 Libraries (µL) includes excess
End-repaired DNA	24	
SureSelect XT S4 P1 (purple cap)*	26	325
SureSelect XT S4 IA (blue cap)*	26	325
5X T4 DNA Ligase buffer†	20	250
T4 DNA Ligase (red cap)†	1.5	18.75
Nuclease-free water	2.5	31.25
Total	**100**	**950 (76 µL/sample)**

* Included in the SureSelect LT Barcoding Lib. Kit. Note that adaptors provided in the SureSelect LT Barcoding Lib. Kit are truncated compared to the adaptors provided in the SOLiD Fragment Library Oligos Kit and are not interchangeable.

† Included in the SureSelect Library Prep Kit, S4.

3 Incubate at room temperature for 15 minutes.

Step 6. Purify the sample using the Agencourt AMPure XP beads

1. Let the AMPure XP beads come to room temperature for at least 30 minutes.
2. Mix the reagent well so that the reagent appears homogeneous and consistent in color. *Do not freeze*.
3. Add 180 µL of homogenous AMPure XP beads to a 1.5-mL LoBind tube, and add the ligated DNA library (100 µL). Mix well on a vortex mixer and incubate for 5 minutes.
4. Put the tube in the magnetic stand. Wait for the solution to clear (approximately 3 to 5 minutes).
5. Keep the tube in the magnetic stand. Do not touch the beads while you carefully discard the cleared solution from the tubes.
6. Continue to keep the tube in the magnetic stand while you dispense 0.5 mL of 70% ethanol in each tube.

   Use fresh 70% ethanol for optimal result.
7. Let the tube sit for 1 minute to allow any disturbed beads to settle, and remove the ethanol.

   Use fresh 70% ethanol for optimal result.
8. Repeat step 6 and step 7 once.
9. Dry the samples on the 37°C heat block for 5 minutes until the residual ethanol is completely evaporated.

   Do not dry the bead pellet to the point that the bead pellet appears cracked. Elution efficiency is significantly decreased when the bead pellet is excessively dried.
10. Add 21 µL nuclease-free water, mix well on a vortex mixer, and incubate for 2 minutes at room temperature.
11. Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
12. Remove the supernatant (~21 µL) to a fresh 1.5-mL LoBind tube. You can discard the beads at this time.

Stopping Point If you do not continue to the next step, store the samples at -20°C.

Step 7. Size-select the DNA fragments with E-gel SizeSelect 2% gel

1. Remove an E-Gel SizeSelect 2% Gels from its package. Remove the combs from the top sample-loading wells and the middle collection wells. Set the E-Gel on the E-Gel iBase linked with the E-Gel Safe Imager.
2. Load the E-Gel as follows:
   a. Into each well in the *top row*, load 20 µL of the ligated, purified DNA. If the sample volume < 20 µL, add nuclease-free water to the well for a total volume of 20 µL. Do not use the center well (smaller well in the top center of the gel for the ladder) or the two outermost wells (to avoid edge effects).
   b. Load 2 µL 50-bp ladder at 0.1 µg/µL to the center top well. Add 15 µL of water to fill the well.
   c. Fill empty wells in the top row with 20 µL of nuclease-free water.
   d. Fill each of the collection wells in the *middle* of the gel with 25 µL of nuclease-free water. Add 20 µL of nuclease-free water to the *middle* center well.
3. Run the gel:
   - iBase program: **Run E-Gel DC**
   - Approximate run time: **13:45** (13 minutes and 45 seconds)

   Monitor the E-Gel in real-time with the E-Gel® Safe Imager.
4. If needed during the run, fill the middle collection wells with nuclease-free water.
5. When the 200-bp band from the marker lane is in the center of the collection well, stop the run if the run has not already stopped (see Figure 3).
6. Collect the solution from the wells.
7. Wash each collection well with 25 µL of nuclease-free water, pipette up and down, then retrieve the wash solution and combine with the respective sample solution collected in step 6.

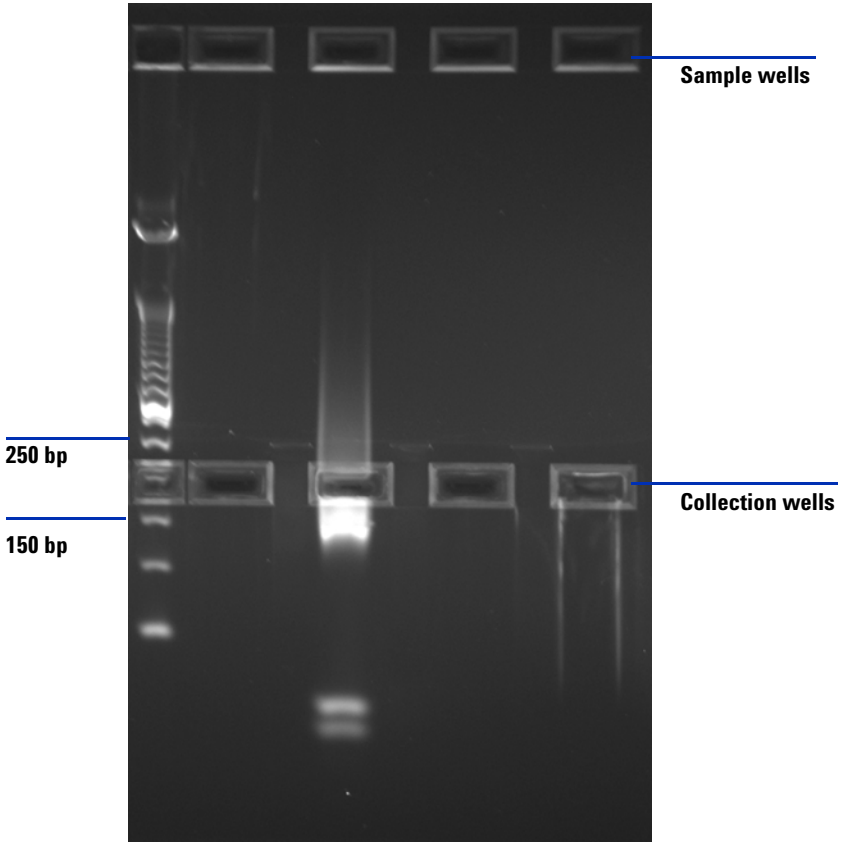

Figure 3 Elution of an approximately 200 bp region from E-gel SizeSelect 2% gel.

Step 8. Amplify the library

Use reagents from the SureSelect LT Barcoding Lib. Kit and the Herculase II Fusion DNA Polymerase (Agilent).

1 Prepare a master mix for the number of reactions needed, plus one additional reaction for the negative control, in Table 14.

Table 14 Amplify the gel eluate

Component	Volume for 1 Library	Volume for 12 Libraries (include excess)
SureSelect XT S4 Pre Capture Primer (green cap)*	8 µL	100 µL
5X Herculase II Rxn Buffer (clear cap)†	40 µL	500 µL
100 mM dNTP Mix (green cap)†	2 µL	25 µL
Herculase II Fusion DNA Polymerase (red cap)†	4 µL	50 µL
Nuclease-free water	116 µL	1450 µL
Total	**170 µL**	**2125 µL**

* Included in the SureSelect LT Barcoding Lib. Kit

† Included in the Herculase II Fusion DNA Polymerase (Agilent) kit

2 Add 170 µL of master mix to 30 µL of the gel eluate, then distribute into 4 PCR reaction tubes.

3 Run the PCR program in Table 15.

Table 15 PCR conditions to amplify the library

Stage	Step	Temp	Time
Holding	Denature	98°C	30 sec
Cycling (12 cycles)	Denature	98°C	10 sec
	Anneal	54°C	30 sec
	Extend	72°C	30 sec
Holding	Extend	72°C	2 min
Holding	—	4°C	∞

4 Pool respective PCR samples into a new 1.5-mL LoBind tube.

Step 9. Purify the sample using the Agencourt AMPure XP beads

1. Let the AMPure XP beads come to room temperature for at least 30 minutes.
2. Mix the reagent well so that the reagent appears homogeneous and consistent in color. Do not freeze.
3. Add 360 µL of homogenous AMPure XP beads to a 1.5-mL LoBind tube, and add the amplified library (200 µL). Mix well on a vortex mixer and incubate for 5 minutes.
4. Put the tube in the magnetic stand. Wait for the solution to clear (approximately 3 to 5 minutes).
5. Keep the tube in the magnetic stand. Do not touch the beads while you carefully discard the cleared solution from the tubes.
6. Continue to keep the tube in the magnetic stand while you dispense 500 µL of 70% ethanol in each tube.

   Use fresh 70% ethanol for optimal result.
7. Let the tube sit for 1 minute to allow any disturbed beads to settle, and remove the ethanol.
8. Repeat step 6 and step 7 once.
9. Dry the samples on the 37°C heat block for 5 minutes until the residual ethanol completely evaporates.

   Do not dry the bead pellet to the point that the bead pellet appears cracked. Elution efficiency is significantly decreased when the bead pellet is excessively dried.
10. Add 50 µL nuclease-free water, mix well on a vortex mixer, and incubate for 2 minutes at room temperature.
11. Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
12. Remove approximately 50 µL of the supernatant to a fresh 1.5-mL LoBind tube. You can discard the beads at this time.

Step 10. Quantify the Library with the 2100 Bioanalyzer DNA 1000 assay

NOTE As an alternative, you can use the D1K ScreenTape (Agilent p/n 5067-5361) and D1K Reagents (Agilent p/n 5067-5362). For more information to do this step, see the *Agilent 2200 TapeStation User Manual*.

1. Check that the 2100 Bioanalyzer electrodes have been cleaned and dried as instructed in the reagent kit guide.
2. Open the Agilent 2100 Expert Software (version B.02.07 or higher), turn on the 2100 Bioanalyzer and check communication.
3. Prepare the chip, samples and ladder as instructed in the reagent kit guide, then mix on the IKA vortex mixer that is included with the 2100 Bioanalyzer.
4. Load the prepared chip into the 2100 Bioanalyzer and start the run within five minutes after preparation.
5. Within the instrument context, choose the appropriate assay from the drop down list.
6. Start the run. Enter sample names and comments in the Data and Assay context.
7. Verify the results. Check that the electropherogram shows a distribution with a peak size around 200 bp ± 10%.

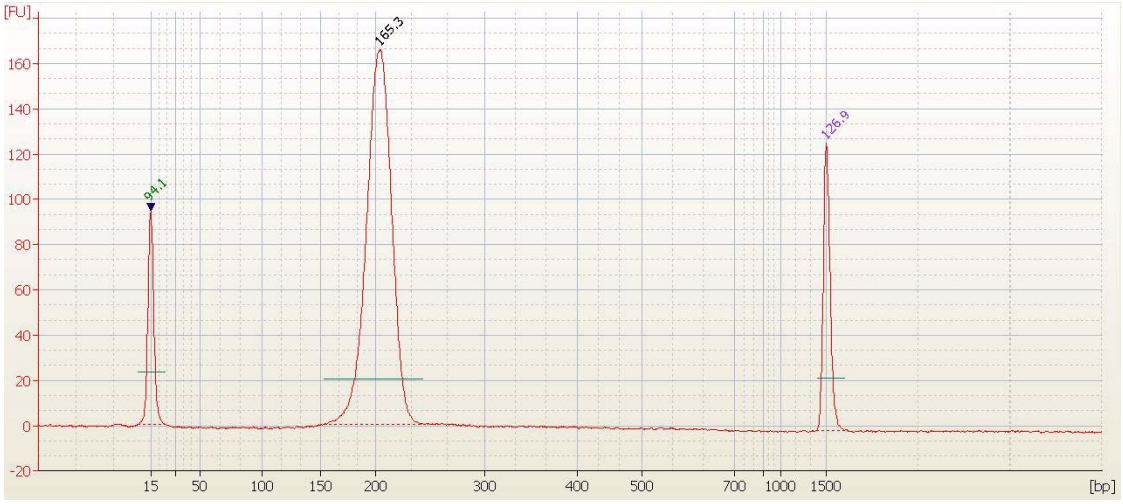

Figure 4 Analysis of amplified prepped library DNA using a DNA 1000 assay. The electropherogram shows a single peak in the size range of approximately 200 bp ± 10%.

8 If the concentration of your sample is greater than the high end of the dynamic range of the Bioanalyzer DNA 1000 assay (> 50 ng/µL), use the Qubit Fluorometer to quantitate the library. Dilute your prepped library appropriately, and use the Bioanalyzer DNA 1000 assay to quantitate again. Use the concentration as determined by the Bioanalyzer DNA 1000 assay to calculate the volume of prepped library needed for hybridization (500 ng) in Chapter 3.

Stopping Point If you do not continue at the next step, store the purified DNA in RNase-free water at 4°C.

3 Hybridization

Step 1. Hybridize the library 36
Step 2. Prepare magnetic beads 42
Step 3. Select hybrid capture with SureSelect 43
Step 4. Purify the sample using Agencourt AMPure XP beads 45

This chapter describes the steps to combine the prepped library with the hybridization reagents, blocking agents and the SureSelect capture library.

CAUTION The ratio of SureSelect capture library to prepped library is critical for successful capture.

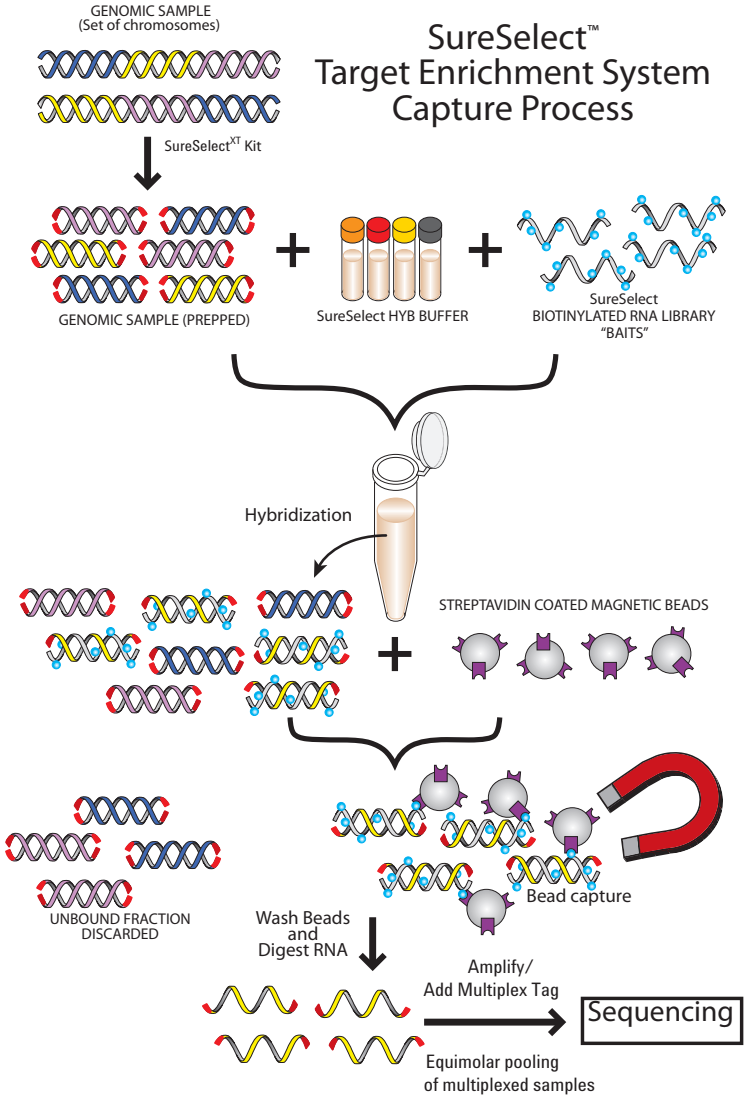

Figure 5 SureSelect Target Enrichment System Capture Process

Refer to "SureSelect Reagent Kit Content" on page 60 for a complete content listing of each SureSelect Target Enrichment kit.

CAUTION

You must avoid evaporation from the small volumes of the capture during the 24 hour or greater incubation.

If you want to use a different combination of thermal cycler, lid temperature, plates or strips, and sealing method (strip caps or sealing tape), first test the conditions. Incubate 27 µL of SureSelect Hybridization Buffer (without DNA) at 65°C for 24 hours (or longer, if applicable) as a test. Include buffer in each well that you might use, including those in the center and those on the edges. Check that you do not get extensive evaporation. Evaporation should not exceed 3 to 4 µL.

For a partial list of tested options showing minimal evaporation, refer to "Alternative Capture Equipment Combinations" on page 64.

Step 1. Hybridize the library

For each sample library prepared, do one hybridization and capture. Do not pool samples at this stage.

The hybridization reaction requires 500 ng of DNA with a maximum volume of 3.4 µL.

1. If the prepped library concentration is below 147 ng/µL, use a vacuum concentrator to concentrate the sample at ≤ 45°C.
   a. Add the entire 50 µL of prepped library to an Eppendorf tube. Poke one or more holes in the lid with a narrow gauge needle.

      You can also break off the cap, cover with parafilm, and poke a hole in the parafilm.

   b. Completely lyophilize. Use a vacuum concentrator on low heat (less than 45°C) to dehydrate.

   c. Reconstitute with nuclease-free water to bring the final concentration to 147 ng/µL (or greater if sample recovery is of concern). Pipette up and down along the sides of the tube for optimal recovery.

   d. Mix well on a vortex mixer and spin in a microfuge for 1 minute.
2. *Optional.* To test recovery after lyophilization, reconstitute the sample to greater than 147 ng/µL and check the concentration on a Bioanalyzer DNA 1000 chip. See "Step 10. Quantify the Library with the 2100 Bioanalyzer DNA 1000 assay" on page 31. After quantitation, adjust the sample to 147 ng/µL.

   Alternatively, concentrate a 500 ng aliquot at ≤ 45°C down to 3.4 µL. If the sample dries up completely, resuspend in 3.4 µL of water and mix on a vortex mixer. If processing multiple samples, adjust to equivalent volumes before concentrating.
3. Mix the components in Table 16 at room temperature to prepare the hybridization buffer.

Table 16 Hybridization Buffer

Reagent	Volume for 1 capture (µL), includes excess	Volume for 6 captures (µL), includes excess	Volume for 12 captures (µL), includes excess
SureSelect Hyb #1 (orange cap, or bottle)	25	125	250
SureSelect Hyb #2 (red cap)	1	5	10
SureSelect Hyb #3 (yellow cap)	10	50	100
SureSelect Hyb #4 (black cap, or bottle)	13	65	130
Total	**49 (40 µL needed)**	**245 (40 µL/sample)**	**490 (40 µL/sample)**

4 If precipitate forms, warm the hybridization buffer at 65°C for 5 minutes.

5 In a PCR plate, prepare the SureSelect capture library mix for target enrichment:

- **a** Keep tubes on ice until step 10.
- **b** For each sample, add the amount of SureSelect capture library as listed in Table 17, based on the Mb target size of your design.
- **c** Use nuclease-free water to prepare a dilution of the SureSelect RNase Block (purple cap) as listed in Table 17.

  Prepare enough RNase Block dilution for all samples, plus excess.
- **d** Add the amount of diluted SureSelect RNase Block (purple cap) listed in Table 17 to each capture library, and mix by pipetting.

Table 17 SureSelect Capture Library.

Capture Size	Volume of SureSelect Library	RNase Block Dilution (Parts RNase block: Parts water)	Volume of RNase Block Dilution to Add
< 3.0 Mb	2 µL	1:6	5 µL
≥ 3.0 Mb	5 µL	1:2	2 µL

6 Mix the contents in Table 18 to make the correct amount of SureSelect Block mix for the number of samples used.

Table 18 SureSelect Block Mix

Reagent	Volume for 1 reaction	Volume for 12 reactions (includes excess)
SureSelect Indexing Block #1 (green cap)	2.5 µL	31.25 µL
SureSelect Block #2 (blue cap)	2.5 µL	31.25 µL
SureSelect LT Indexing Block #3 (brown cap)	0.6 µL	7.5 µL
Total	**5.6 µL**	**70 µL**

7 In a separate PCR plate, prepare the prepped library for target enrichment.

- **a** Add 3.4 µL of 147 ng/µL prepped library to the "B" row in the PCR plate. Put each sample into a separate well.
- **b** Add 5.6 µL of the SureSelect Block Mix to each well in row B.
- **c** Mix by pipetting up and down.
- **d** Seal the wells of row "B" with caps and put the PCR plate in the thermal cycler. Do not heat the Hybridization Buffer or capture library yet, only the prepped library with blockers.
- **e** Start the thermal cycler program in Table 19.

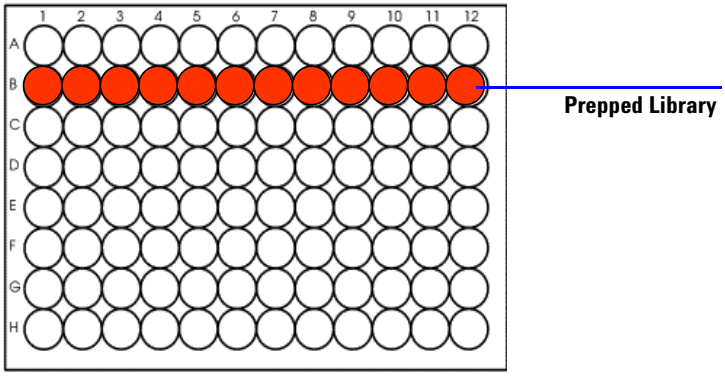

Figure 6 Prepped library shown in red

Table 19 PCR program

Step	Temperature	Time
Step 1	95°C	5 minutes
Step 2	65°C	Hold

8 Use a heated lid on the thermal cycler at 105°C to hold the temperature of the plate on the thermal cycler at 65°C.

CAUTION The lid of the thermal cycler is hot and can cause burns. Use caution when working near the lid.

9 Maintain the plate at 65°C while you load 40 µL of hybridization buffer per well into the "A" row of the PCR plate. The number of wells filled in Row A is the number of libraries prepared.

The example in Figure 7 is for 12 captures.

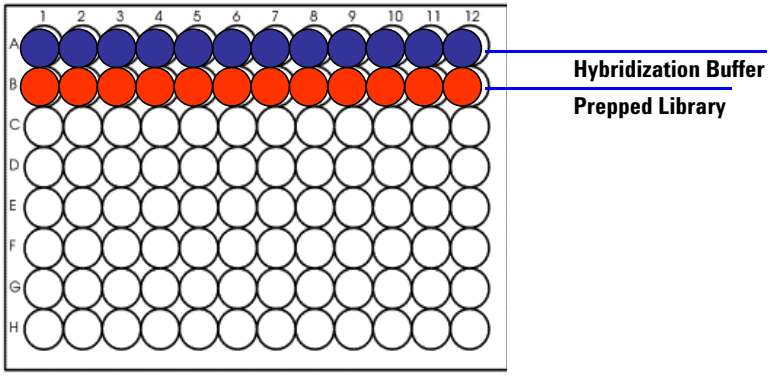

Figure 7 Hybridization buffer shown in blue

Make sure that the plate is at 65°C for a minimum of 5 minutes before you go to step 10.

10 Add the capture library mix from step 5 to the PCR plate:

a Add the capture library mix (7 µL) to the "C" row in the PCR plate.

For multiple samples, use a multi-channel pipette to load the capture library mix into the "C" row in the PCR plate.

Keep the plate at 65°C during this time.

b Seal the wells with strip caps. Use a capping tool to make sure the fit is tight.

c Incubate the samples at 65°C for 2 minutes.

11 Maintain the plate at 65°C while you use a multi-channel pipette to take 13 µL of Hybridization Buffer from the "A" row and add it to the SureSelect capture library mix contained in row "C" of the PCR plate for each sample. (See Figure 8.)

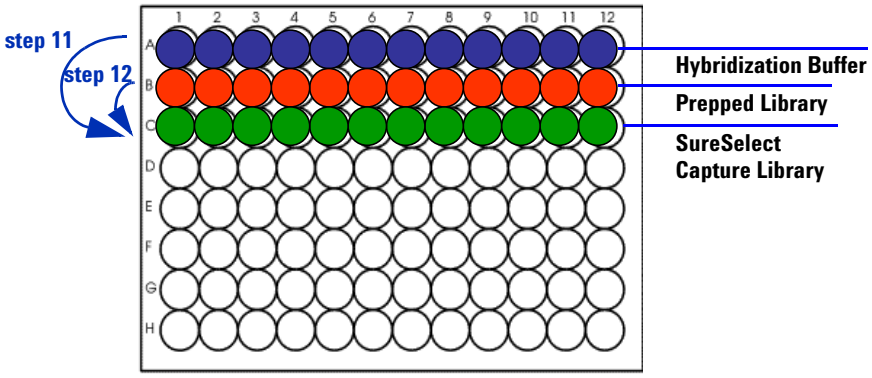

Figure 8 SureSelect Capture Library, or "Baits", shown in Green

12 Maintain the plate at 65°C while you use a multi-channel pipette to transfer the entire contents of each prepped library mix in row "B" to the hybridization solution in row "C". (See Figure 8.) Mix well by slowly pipetting up and down 8 to 10 times.

The hybridization mixture is now 27 to 29 µL, depending on degree of evaporation during the preincubations.

13 Seal the wells with strip caps or double adhesive film. Make sure all wells are completely sealed.

Use new adhesive seals or strip caps. The structural integrity of the seals and caps can be compromised during the previous incubation steps.

CAUTION Wells must be adequately sealed to minimize evaporation, or your results can be negatively impacted.

If you use strip tubes, test for evaporation before you do the first experiment to make sure the tube/capping method is appropriate for the thermal cycler. Check that no more than 3 to 4 µL is lost to evaporation.

14 Incubate the hybridization mixture for 24 hours at 65°C with a heated lid at 105°C.

Step 2. Prepare magnetic beads

Use these reagents from the SureSelect Target Enrichment Kit Box #1:

- SureSelect Binding Buffer
- SureSelect Wash 2

1. Prewarm SureSelect Wash 2 at 65°C in a circulating water bath for use in "Step 3. Select hybrid capture with SureSelect".
2. Vigorously resuspend the Dynabeads MyOne Streptavidin T1 on a vortex mixer. Magnetic beads settle during storage.
3. For each hybridization, add 50 µL of Dynabeads MyOne Streptavidin T1 to a 1.5-mL LoBind tube.
4. Wash the beads:
   a. Add 200 µL of SureSelect Binding Buffer.
   b. Mix the beads on a vortex mixer for 5 seconds.
   c. Put the tubes into a magnetic device, such as the Dynal magnetic separator (Life Technologies).
   d. Remove and discard the supernatant.
   e. Repeat step a through step d for a total of 3 washes.
5. Resuspend the beads in 200 µL of SureSelect Binding Buffer.

Step 3. Select hybrid capture with SureSelect

Use these reagents from the SureSelect Target Enrichment Kit Box #1:

- SureSelect Wash 1
- SureSelect Wash 2
- SureSelect Elution Buffer
- SureSelect Neutralization Buffer

CAUTION Keep the Elution Buffer container tightly sealed when not in use. Prolonged exposure of SureSelect Elution Buffer to air can decrease product performance by altering the pH of the solution.

1. Estimate and record the volume of hybridization that remained after 24 hour incubation.
2. Keep the PCR plate or tubes at 65°C in the PCR machine while you add the hybridization mixture directly from the thermal cycler to the bead solution. Invert the tube to mix 3 to 5 times.

   Excessive evaporation, such as when less than 20 µL remains after hybridization, can indicate suboptimal capture performance. See Table 34 on page 64 for tips to minimize evaporation.
3. Incubate the hybrid-capture/bead solution on a Nutator or equivalent for 30 minutes at room temperature.

   Make sure the sample is properly mixing in the tube.
4. Briefly spin in a centrifuge.
5. Separate the beads and buffer on a magnetic separator and remove the supernatant.
6. Resuspend the beads in 500 µL of SureSelect Wash 1 by mixing on a vortex mixer for 5 seconds.
7. Incubate the samples for 15 minutes at room temperature.
8. Briefly spin in a centrifuge.
9. Separate the beads and buffer on a magnetic separator and remove the supernatant.

10 Wash the beads:

- **a** Resuspend the beads in 500 µL of 65°C prewarmed SureSelect Wash 2 and mix on a vortex mixer for 5 seconds to resuspend the beads.
- **b** Incubate the samples for 10 minutes at 65°C in a recirculating water bath, heat block or equivalent.

  Do not use a tissue incubator. It cannot properly maintain temperature.
- **c** Briefly spin in a centrifuge.
- **d** Separate the beads and buffer on a magnetic separator and remove the supernatant.
- **e** Repeat step a through step d for a total of 3 washes.

  Make sure all of the wash buffer has been removed.

11 Mix the beads in 50 µL of SureSelect Elution Buffer on a vortex mixer for 5 seconds to resuspend the beads.

12 Incubate the samples for 10 minutes at room temperature.

13 Briefly spin in a centrifuge.

14 Separate the beads and buffer on a magnetic separator.

15 Use a pipette to transfer the supernatant to a new 1.5-mL LoBind tube.

The supernatant contains the captured DNA. The beads can now be discarded.

16 Add 50 µL of SureSelect Neutralization Buffer to the captured DNA.

17 Briefly mix on a vortex mixer.

Step 4. Purify the sample using Agencourt AMPure XP beads

1. Let the AMPure XP beads come to room temperature for at least 30 minutes.
2. Mix the reagent well so that the reagent appears homogeneous and consistent in color. *Do not freeze*.
3. Add 180 µL of homogenous AMPure XP beads to a 1.5-mL LoBind tube, and add the 100 µL of captured DNA library. Mix well on a vortex mixer and incubate for 5 minutes.
4. Put the tube in the magnetic stand. Wait for the solution to clear (approximately 3 to 5 minutes).
5. Keep the tube in the magnetic stand. Do not touch the beads while you carefully discard the cleared solution from the tubes.
6. Continue to keep the tube in the magnetic stand while you dispense 0.5 mL of 70% ethanol in each tube.

   Use fresh 70% ethanol for optimal result.
7. Let the tube sit for 1 minute to allow any disturbed beads to settle, and remove the ethanol.
8. Repeat step 6 and step 7 step once.
9. Dry the samples on the 37°C heat block for 5 minutes or until the residual ethanol completely evaporates.

   Do not dry the bead pellet to the point that the bead pellet appears cracked. Elution efficiency is significantly decreased when the bead pellet is excessively dried.
10. Add 50 µL nuclease-free water, mix well on a vortex mixer, and incubate for 2 minutes at room temperature.
11. Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
12. Remove the supernatant (~50 µL) to a fresh 1.5-mL LoBind tube. You can discard the beads at this time.

Stopping Point If you do not continue to the next step, store the samples at -20°C in TE for longer periods.

4 Addition of Barcode Tags by Post-Hybridization Amplification

Step 1. Amplify the captured library to add barcode tags 48
Step 2. Purify the sample using Agencourt AMPure XP beads 52
Step 3. Assess quality with the 2100 Bioanalyzer High Sensitivity DNA assay 53
Step 4. Assess the quantity of each barcode-tagged library by QPCR 56
Step 5. Pool samples for Multiplexed Sequencing 57
Step 6. Do an emulsion PCR 58

This chapter describes the steps to add barcode tags by amplification, purify, assess quality and quantity of the libraries, and pool barcoded samples for multiplexed sequencing.

Step 1. Amplify the captured library to add barcode tags

Use reagents from:

- Herculase II Fusion DNA Polymerase (Agilent)
- SureSelect LT Barcoding Lib. Kit or SureSelect Library Prep Kit, S4

CAUTION Do not use amplification enzymes other than Herculase II Fusion DNA Polymerase. Other enzymes have not been validated.

CAUTION To avoid cross-contaminating libraries, set up PCR reactions (all components except the library DNA) in a dedicated clean area or PCR hood with UV sterilization and positive air flow.

Prepare 1 amplification reaction for each hybrid capture. Include a negative no-template control.

To see the nucleotide sequence in each of the barcode included in SureSelect reagent kits, see "SureSelect^{XT} Barcodes for SOLiD" on page 63.

1 For 1 library:
   - In a PCR tube, strip tube, or plate, prepare the reaction mix in Table 20, on ice. Mix well by gently pipetting up and down.

2 For multiple libraries:
   a Prepare the reaction mix in Table 20, on ice. Mix well on a vortex mixer.
   b Add 46 µL of the reaction mix to each well or tube.
   c Add 4 µL of the appropriate barcode SureSelect XT LT BC1 through BC16 (clear cap) from the SureSelect LT Barcoding Lib. Kit or SureSelect Library Prep Kit, S4 to each well and mix by pipetting.

      Use Table 21 as a guide to determine the number of barcodes to pool per sequencing lane.

      For each multiplexed sequencing run, use at least one of the following full sets of four barcodes: Barcodes BC1–4, BC5–8, BC9–12, BC13–16. If fewer than four samples are to be prepared for sequencing, apply multiple barcodes per sample in equal ratios. For example if only three samples are prepared, apply BC1 to sample 1 and BC2 to sample 2. Mix 2 µL of BC3 with 2 µL of BC4 and apply that mix to sample 3.

d Use a pipette to add 50 µL of each DNA sample to each well or tube. Mix by pipetting. Change pipette tips between samples to avoid cross-contamination.

Table 20 Herculase II Master Mix

Reagent	Volume for 1 reaction	Volume for 12 reactions (includes excess)
Captured DNA	50 µL	
Nuclease-free water	23 µL	287.5 µL
5X Herculase II Rxn Buffer (clear cap)*	20 µL	250 µL
100 mM dNTP Mix (green cap)*	1 µL	12.5 µL
Herculase II Fusion DNA Polymerase (red cap)*	2 µL	25 µL
SureSelect XT LT BC1 through BC16 (clear cap)	4 µL†	
Total	**100 µL**	**575 µL (46 µL/reaction)**

* Included in the Herculase II Fusion DNA Polymerase (Agilent). *Do not use the buffer or dNTP mix from any other kit.*

† Included in the SureSelect LT Barcoding Lib. Kit or SureSelect Library Prep Kit, S4. For 1 reaction, use one full set of four barcodes: Barcodes BC1-4, BC5-8, BC9-12, or BC13-16. Use 1 uL of each barcode in the set.

Table 21 Sequencing data requirement guidelines

Capture size	Optimal sequencing output per barcode
1 kb up to 0.5 Mb	0.1 to 50 Mb*
0.5 Mb up to 2.9 Mb	50 to 290 Mb*
3 Mb up to 5.9 Mb	300 to 590 Mb*
6 Mb up to 11.9 Mb	600 to 1190 Mb*
12 Mb up to 24 Mb	1.2 to 2.4 Gb*
Human All Exon v4	4 Gb
Human All Exon v4 + UTRs	6 Gb
Human All Exon 50 Mb	5 Gb
Human DNA Kinome	320 Mb
Mouse All Exon	5 Gb

* For custom libraries, Agilent recommends analyzing 100× amount of sequencing data compared to the Capture Library size for each sample. Pool samples according to your expected sequencing output.

3 Put the tubes in a thermal cycler and run the program in Table 22.

Table 22 PCR program

Step	Temperature	Time
Step 1	95°C	5 minutes
Step 2	95°C	15 seconds
Step 3	54°C	45 seconds
Step 4	70°C	1 minute
Step 5		Repeat Step 2 through Step 4, depending on the size of the capture: • 0.2 Mb up to 0.49 Mb: 12 cycles total • 0.5 Mb up to 1.49 Mb: 10 cycles total • 1.5 Mb up to 2.99 Mb: 9 cycles total • 3 Mb or more: 8 cycles total
Step 6	70°C	5 minutes
Step 7	4°C	Hold

Step 2. Purify the sample using Agencourt AMPure XP beads

1. Let the AMPure XP beads come to room temperature for at least 30 minutes.
2. Mix the reagent well so that the reagent appears homogeneous and consistent in color. *Do not freeze*.
3. Add 180 µL of homogenous AMPure beads to a 1.5-mL LoBind tube, and add amplified library (~100 µL). Mix well on a vortex mixer and incubate for 5 minutes.
4. Put the tube in the magnetic stand. Wait for the solution to clear (approximately 3 to 5 minutes).
5. Keep the tube in the magnetic stand. Do not touch the beads while you carefully discard the cleared solution from the tubes.
6. Continue to keep the tube in the magnetic stand while you dispense 500 µL of 70% ethanol in each tube.

   Use fresh 70% ethanol for optimal result.
7. Let the tube sit for 1 minute to allow any disturbed beads to settle, and remove the ethanol.
8. Repeat step 6 and step 7 once.
9. Dry the samples on the 37°C heat block for 5 minutes or until the residual ethanol is completely evaporated.

   Do not dry the bead pellet to the point that the bead pellet appears cracked. Elution efficiency is significantly decreased when the bead pellet is excessively dried.
10. Add 30 µL nuclease-free water, mix well on a vortex mixer, and incubate for 2 minutes at room temperature.
11. Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
12. Remove the supernatant (~30 µL) to a fresh 1.5-mL LoBind tube. You can discard the beads at this time.

Stopping Point If you do not continue to the next step, store the samples at 4°C for up to a week, or at -20°C for longer periods.

Step 3. Assess quality with the 2100 Bioanalyzer High Sensitivity DNA assay

Use a Bioanalyzer High Sensitivity DNA Assay to assess the quality and size range. You may need to dilute your sample accordingly. Refer to the *Agilent High Sensitivity DNA Kit Guide* at http://www.chem.agilent.com/en-US/Search/Library/_layouts/Agilent/PublicationSummary.aspx?whid=59504.

1. Check that the 2100 Bioanalyzer electrodes have been cleaned as instructed in the reagent kit guide.
2. Open the Agilent 2100 Expert Software (version B.02.07 or higher required to run the High Sensitivity Kit), turn on the 2100 Bioanalyzer and check communication.
3. Prepare the chip, samples and ladder as instructed in the reagent kit guide.
4. Load the prepared chip into the 2100 Bioanalyzer and start the run within five minutes after preparation.
5. Within the instrument context, choose the appropriate assay from the drop down list.
6. Start the run. Enter sample names and comments in the Data and Assay context.
7. Verify the results.

   Determine the concentration of the sample by integration under the peak.
8. If primer-dimers are observed in the 50 bp to 75 bp size range:
   a. Add 20 µL of nuclease-free water to your sample.
   b. Repeat "Step 2. Purify the sample using Agencourt AMPure XP beads" on page 52 with 90 µL of homogenous AMPure XP Beads.
   c. Repeat "Step 3. Assess quality with the 2100 Bioanalyzer High Sensitivity DNA assay" on page 53.

9 If amplification products are still not visible:

a Add the components in Table 20 on page 49 with the entire amount of eluate from "Step 2. Purify the sample using Agencourt AMPure XP beads" on page 52.

b Run the PCR cycling program in Table 23.

c Repeat "Step 2. Purify the sample using Agencourt AMPure XP beads" on page 52.

d Run another Bioanalyzer High Sensitivity DNA assay to confirm and quantitate DNA amplification.

e Repeat step a through step d if needed, until amplification is observed.

Do not overamplify the sample. When you see even trace amplification, continue at "Step 6. Do an emulsion PCR" on page 58.

Table 23 PCR program

Step	Temperature	Time
Step 1	95°C	5 minutes
Step 2	95°C	15 seconds
Step 3	54°C	45 seconds
Step 4	70°C	1 minute
Step 5		Repeat Step 2 through Step 4 for a total of 2 times.
Step 6	70°C	5 minutes
Step 7	4°C	Hold

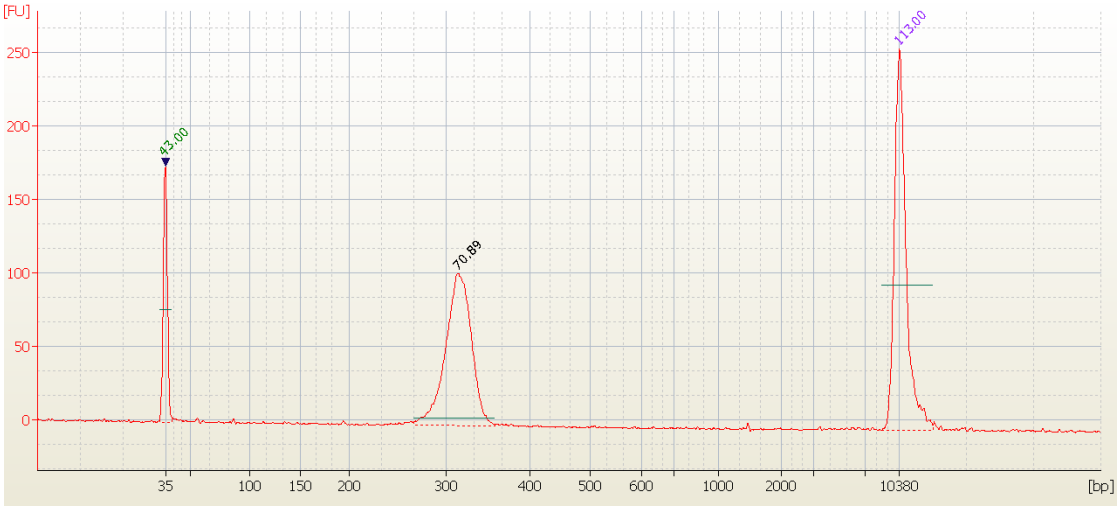

Figure 9 Analysis of Amplified Capture DNA using the High Sensitivity DNA Kit. The electropherogram shows a peak in the size range of approximately 270 to 350 nucleotides.

Step 4. Assess the quantity of each barcode-tagged library by QPCR

Refer to the protocol that is included with the QPCR NGS Library Quantification Kit (SOLiD) for more details to do this step.

1. Use the QPCR NGS Library Quantification Kit (SOLiD) to determine the concentration of each barcode-tagged captured library.
2. Prepare a standard curve using the quantification standard included in the kit, according to the instructions provided in the user guide.
3. Dilute each barcode-tagged captured library such that it falls within the range of the standard curve.

   Typically this corresponds to approximately a 1:1000 to 1:10,000 dilution of the captured DNA.
4. Prepare the QPCR master mix with SOLiD adaptor-specific PCR primers according to instructions provided in the kit.
5. Add an aliquot of the master mix to PCR tubes and add template.
6. On a QPCR system, such as the MX3005P, run the thermal profile outlined in the QPCR NGS Library Quantification kit user guide. Use the SYBR Green instrument setting.
7. Use the standard curve to determine the concentration of each unknown barcode-tagged library, in nM.

The concentration will be used to accurately pool samples for multiplexed sequencing.

Step 5. Pool samples for Multiplexed Sequencing

1 Combine the libraries such that each barcode-tagged sample is present in equimolar amounts in the pool. For each library, use the formula below to determine the amount of barcoded sample to use.

$$\text{Volume of Barcoded Sample} = \frac{V(f) \times C(f)}{\# \times C(i)} \text{ where}$$

V(f) is the final desired volume of the pool,

C(f) is the desired final concentration of all the DNA in the pool, for example, 500 pM for the standard SOLiD protocol

is the number of samples to be combined, and

C(i) is the initial concentration of each barcoded sample.

See Table 24 for the approximate volume of sample to use.

Table 24 Approximate volume of sample to use

SOLiD Sequencing Capacity	Approximate Sample Volume Needed	Final Concentration Needed
Octet	20 µL	500 pM
Quad	20 µL	500 pM
Full Slide	50 to 100 µL	500 pM

Table 25 shows an example of the amount of 4 barcoded samples (of different concentrations) and Low TE needed for a final volume of 100 µL at 500 pM.

Table 25 Example of barcode volume calculation for a total volume of 100 µL

Component	V(f)	C(i)	C(f)	#	Volume to use (µL)
Sample 1	100 µL	921 pM	500 pM	4	13.6
Sample 2	100 µL	1050 pM	500 pM	4	11.9
Sample 3	100 µL	1352 pM	500 pM	4	9.2
Sample 4	100 µL	684 pM	500 pM	4	18.3
Low TE					47

2 Adjust the final volume of the pooled library to the desired final concentration.
   - If the final volume of the combined barcode-tagged samples is less than the desired final volume, V(f), add Low TE to bring the volume to the desired level.
   - If the final volume of the combined barcode-tagged samples is greater than the final desired volume, V(f), lyophilize and reconstitute to the desired volume.
3 If you store the library before sequencing, add Tween 20 to 0.1% v/v and store at -20°C short term.
4 Proceed to emulsion PCR. Refer to the appropriate SOLiD protocol.

To get > 90% of targeted bases with a read depth of > 20 times, barcode tags can be blended together and sequenced in one quad for 0.2 Mb libraries and 6 barcode tags for 3 Mb libraries.

Step 6. Do an emulsion PCR

- Do an emulsion PCR as described in the *Applied Biosystems SOLiD 3 Plus System Templated Bead Preparation Guide* (p/n 4442695).

Stopping Point If you do not do an emulsion PCR at this time, store in RNase-free water 4°C.

5 Reference

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Other Reagent Kits Content 62
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Alternative Capture Equipment Combinations 64

This chapter contains reference information.

SureSelect Reagent Kit Content

Each SureSelect Reagent Kit contains one or more of each of these individual kits:

Table 26 SureSelect Reagent Kit Contents

Product	Storage Condition	16 Reactions	96 Reactions	480 Reactions
SureSelect Target Enrichment Kit Box #1	Room Temperature	5190-4393	5190-4394	5190-4395
SureSelect Target Enrichment Kit LT Indexing Hyb Module Box #2	-20°C	5190-4458	5190-4459	5190-4460
SureSelect Library Prep Kit, S4	-20°C	5500-0106	5500-0078	5500-0079
Product	**Storage Condition**	**1 × 16**	**7 × 16**	
SureSelect LT Barcoding Lib. Kit*	-20°C	G7508A	G7509A	

* The SureSelect LT Barcoding Lib. Kit is included in only the 16-reactions SureSelect^{XT} Reagent Kit, S4. The 96- and 480-reactions SureSelect Library Prep Kit, S4 already includes the content of this kit.

The content of each of these kits are described in the next tables.

Table 27 SureSelect Target Enrichment Kit Box #1

Kit Component
SureSelect Hyb #1 (orange cap, or bottle)
SureSelect Hyb #2 (red cap)
SureSelect Hyb #4 (black cap, or bottle)
SureSelect Binding Buffer
SureSelect Wash 1
SureSelect Wash 2
SureSelect Elution Buffer
SureSelect Neutralization Buffer

Table 28 SureSelect Target Enrichment Kit LT Indexing Hyb Module Box #2

Kit Component
SureSelect Hyb #3 (yellow cap)
SureSelect Indexing Block #1 (green cap)
SureSelect Block #2 (blue cap)
SureSelect LT Indexing Block #3 (brown cap)
SureSelect RNase Block (purple cap)

Table 29 SureSelect Library Prep Kit, S4*

Kit Component
10X End Repair Buffer (clear cap)
5X T4 DNA Ligase buffer
T4 DNA Ligase (red cap)
T4 DNA Polymerase (purple cap)
T4 Polynucleotide Kinase (orange cap)
dNTP mix (green cap)
Klenow DNA Polymerase (yellow cap)

* The 96- and 480-reactions kits also contain the reagents listed in SureSelect LT Barcoding Lib. Kit

Table 30 SureSelect LT Barcoding Lib. Kit*

Component
SureSelect XT S4 P1 (purple cap)
SureSelect XT S4 IA (blue cap)
SureSelect XT S4 Pre Capture Primer (green cap)
SureSelect XT LT BC1 through BC16 (clear cap)

* For the 96- and 480-reactions kits, these reagents are found in the SureSelect Library Prep Kit, S4

Other Reagent Kits Content

These reagents are from kits other than the SureSelect Reagent kit. Make sure you use only the reagents listed here.

Table 31 Herculase II Fusion DNA Polymerase (Agilent)

Component
DMSO (green cap)
5X Herculase II Rxn Buffer (clear cap)
100 mM dNTP Mix (green cap)
Herculase II Fusion DNA Polymerase (red cap)

Table 32 D1K Reagents (Agilent p/n 5067-5362)

Components
D1K ladder
D1K sample buffer

SureSelect^{XT} Barcodes for SOLiD

The nucleotide sequence of each of the SureSelect^{XT} barcodes is listed in Table 33.

Table 33 SureSelect^{XT} Barcodes 1-16

Barcode Number	Sequence
1	GTGTAAGAGG
2	AGGGAGTGGT
3	ATAGGTTATA
4	GGATGCGGTC
5	GTGGTGTAAG
6	GCGAGGGACA
7	GGGTTATGCC
8	GAGCGAGGAT
9	AGGTTGCGAC
10	GCGGTAAGCT
11	GTGCGACACG
12	AAGAGGAAAA
13	GCGGTAAGGC
14	GTGCGGCAGA
15	GAGTTGAATG
16	GGGAGACGTT

Alternative Capture Equipment Combinations

Table 34 lists combinations of thermal cycler, lid temperature, plates or strips, and sealing method (strip caps or sealing tape) other than those used in this protocol that have shown minimal evaporation.

Refer to this list for additional of equipment combination options for hybridization. Note that minimal evaporation is needed to ensure good capture results.

Table 34 Tested options that show minimal evaporation

PCR Machine	Plate/Strips	Cover	Comments
Agilent Mx3005P QPCR	Mx3000P Strip Tubes (401428)	MX3000P Optical Strip Caps (401425)	Heated Lid
Agilent Mx3005P QPCR	MicroAmp Optical 96-well reaction plate (N801-0560)	MicroAmp Clear Adhesive Film (4306311)	Heated Lid; ABI compression pad (4312639) Use two layers of film.
ABI GeneAmp 9700	MicroAmp Optical 96-well Reaction Plate (N801-0560)	MicroAmp Caps (8caps/strip) (N801-0535)	Heated Lid
ABI Veriti (4375786)	MicroAmp Optical 96-well Reaction Plate (N801-0560)	MicroAmp Clear Adhesive Film (4306311)	Heated Lid; ABI compression pad (4312639) Use two layers of film.
Eppendorf Mastercycler	Eppendorf 8-Tube PCR Tubes	Attached lids	Lid heating set to 75°C
BioRad (MJ Research) PTC-200	Agilent strip tubes 410022 (Mx4000)	Agilent Optical cap 410024 (Mx4000)	Heated Lid
BioRad (MJ Research) PTC-200	Agilent strip tubes 410022 (Mx4000)	Agilent Optical cap 401425 (Mx3000/3005)	Heated Lid
BioRad (MJ Research) PTC-200	Agilent 96-well Plate 410088 (Mx3000/3005)	Agilent Optical cap 401425 (Mx3000/3005)	Heated Lid

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In This Book

This guide contains information to run the SureSelect^{XT} Target Enrichment System for SOLiD 4 Multiplexed Sequencing protocol.

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